Supplementary Information

A screen of plant-based natural products revealed that quercetin prevents amyloid-β uptake in astrocytes as well as resulting astrogliosis and synaptic dysfunction

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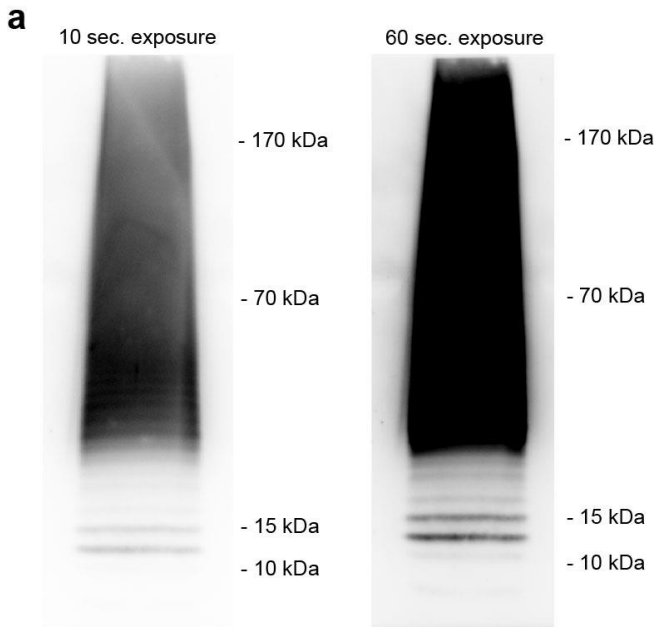
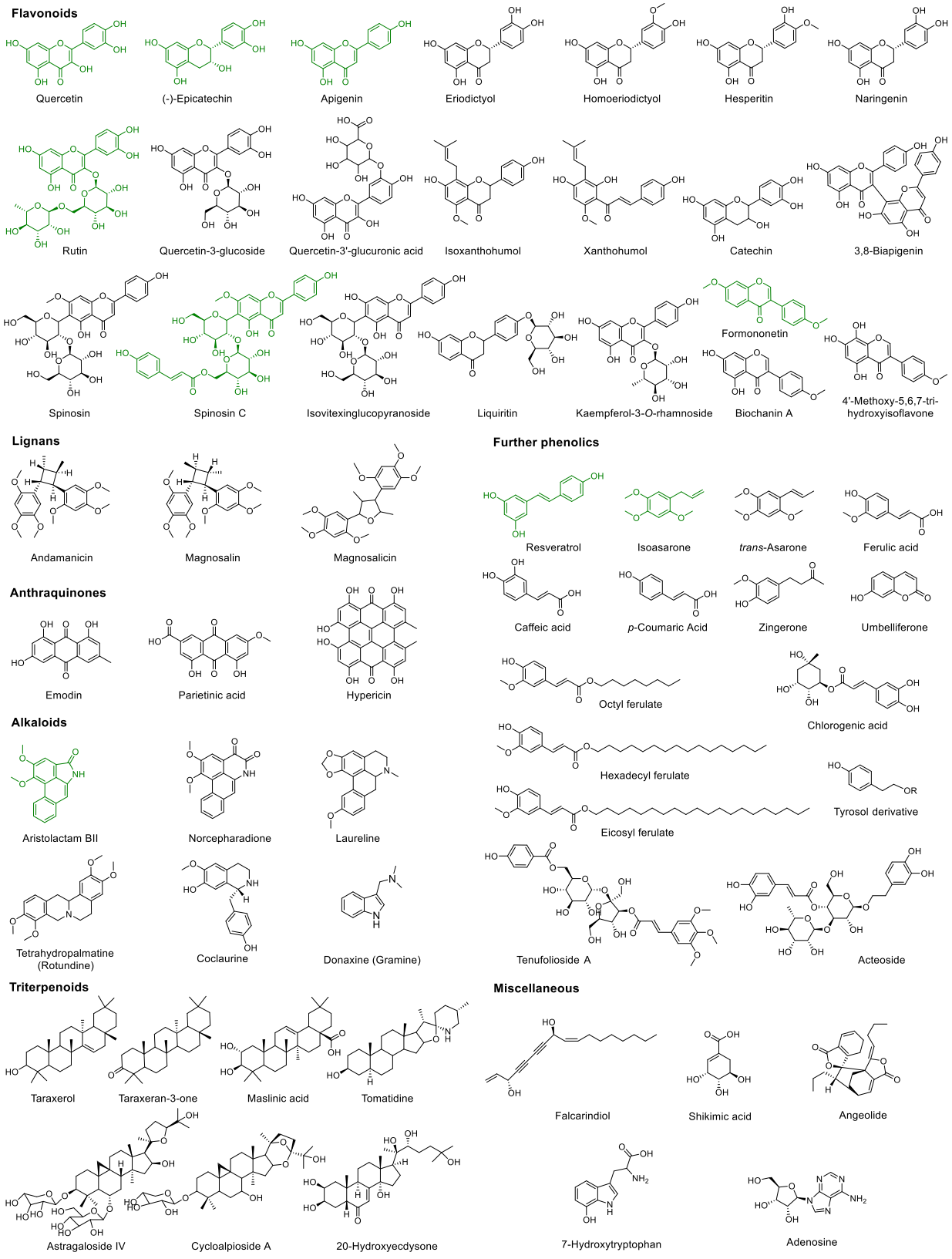


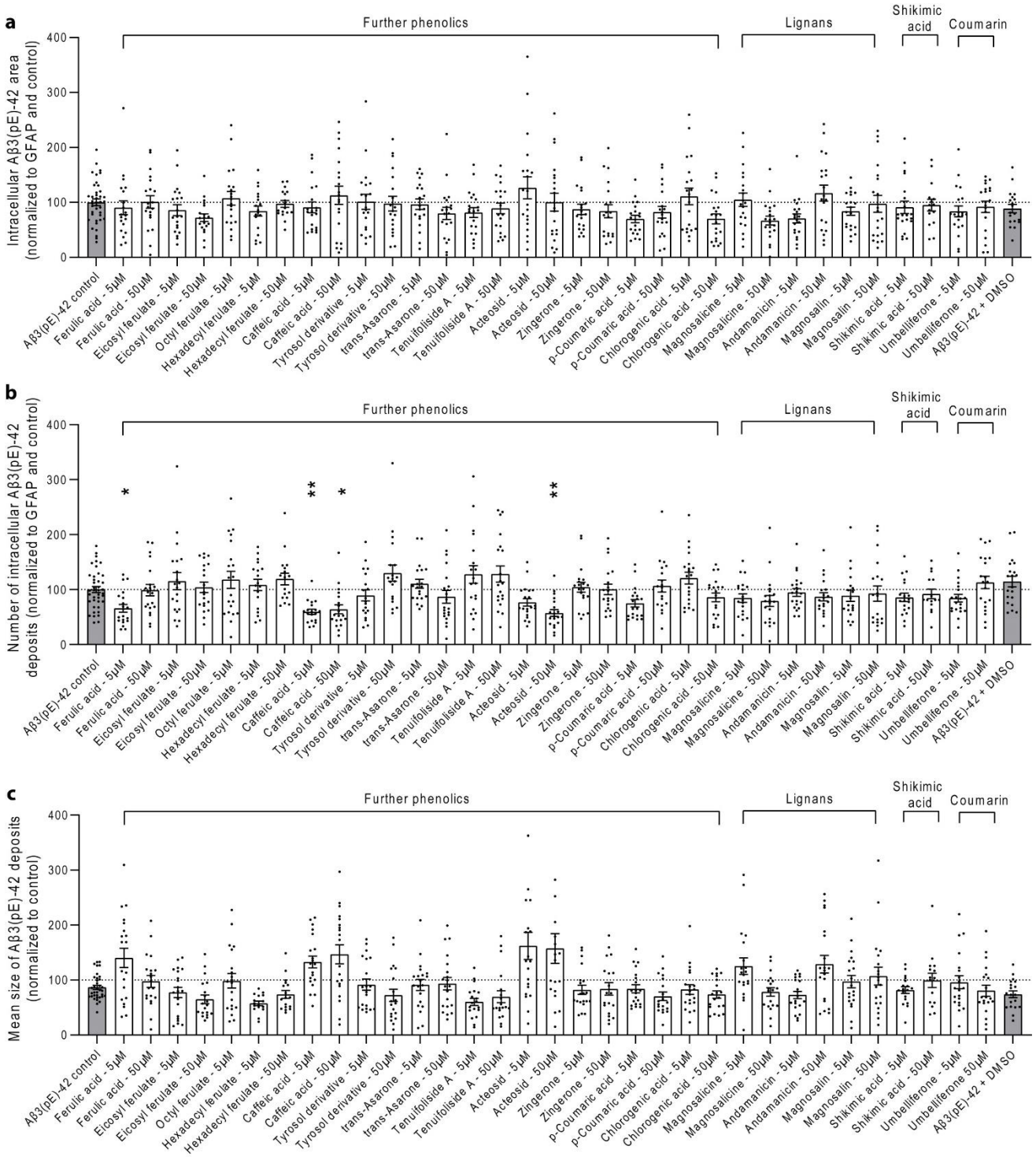
Supplementary Fig. 1 Characterization of oligomeric Aβ3(pE)-42 preparation

(a) SDS-PAGE of Aβ(pE)-42 oligomeric preparation showed protein sizes from di- and trimeric oligomers (8 and 12 kDa) up to larger oligomeric protein complexes (>170 kDa). Depicted 2 Western blot exposure times - 10 sec. and 60 sec.



Supplementary Fig. 2 Structures of tested substances

Green color indicates substances that significantly reduced the uptake of oligomeric Aβ3(pE)-42 to astrocytes.


a
Further phenolics
Lignans
Shikimic acid
Coumarin
Intracellular Aβ3(pE)-42 area (normalized to GFAP and control)
400
300
200
100
0
Aβ3(pE)-42 control
Ferulic acid - 5µM
Ferulic acid - 50µM
Eicosyl ferulate - 5µM
Eicosyl ferulate - 50µM
Octyl ferulate - 5µM
Hexadecyl ferulate - 5µM
Hexadecyl ferulate - 50µM
Caffeic acid - 5µM
Caffeic acid - 50µM
Tyrosol derivative - 5µM
Tyrosol derivative - 50µM
trans-Asarone - 5µM
trans-Asarone - 50µM
Tenuifoliside A - 5µM
Tenuifoliside A - 50µM
Acteosid - 5µM
Acteosid - 50µM
Zingerone - 5µM
Zingerone - 50µM
p-Coumaric acid - 5µM
p-Coumaric acid - 50µM
Chlorogenic acid - 5µM
Chlorogenic acid - 50µM
Magnosalicine - 5µM
Magnosalicine - 50µM
Andamanicin - 5µM
Andamanicin - 50µM
Magnosalin - 5µM
Magnosalin - 50µM
Shikimic acid - 5µM
Shikimic acid - 50µM
Umbelliferone - 5µM
Umbelliferone - 50µM
Aβ3(pE)-42 + DMSO
b
Further phenolics
Lignans
Shikimic acid
Coumarin
Number of intracellular Aβ3(pE)-42 deposits (normalized to GFAP and control)
400
300
200
100
0
*
**
*
**
Aβ3(pE)-42 control
Ferulic acid - 5µM
Ferulic acid - 50µM
Eicosyl ferulate - 5µM
Eicosyl ferulate - 50µM
Octyl ferulate - 5µM
Hexadecyl ferulate - 5µM
Hexadecyl ferulate - 50µM
Caffeic acid - 5µM
Caffeic acid - 50µM
Tyrosol derivative - 5µM
Tyrosol derivative - 50µM
trans-Asarone - 5µM
trans-Asarone - 50µM
Tenuifoliside A - 5µM
Tenuifoliside A - 50µM
Acteosid - 5µM
Acteosid - 50µM
Zingerone - 5µM
Zingerone - 50µM
p-Coumaric acid - 5µM
p-Coumaric acid - 50µM
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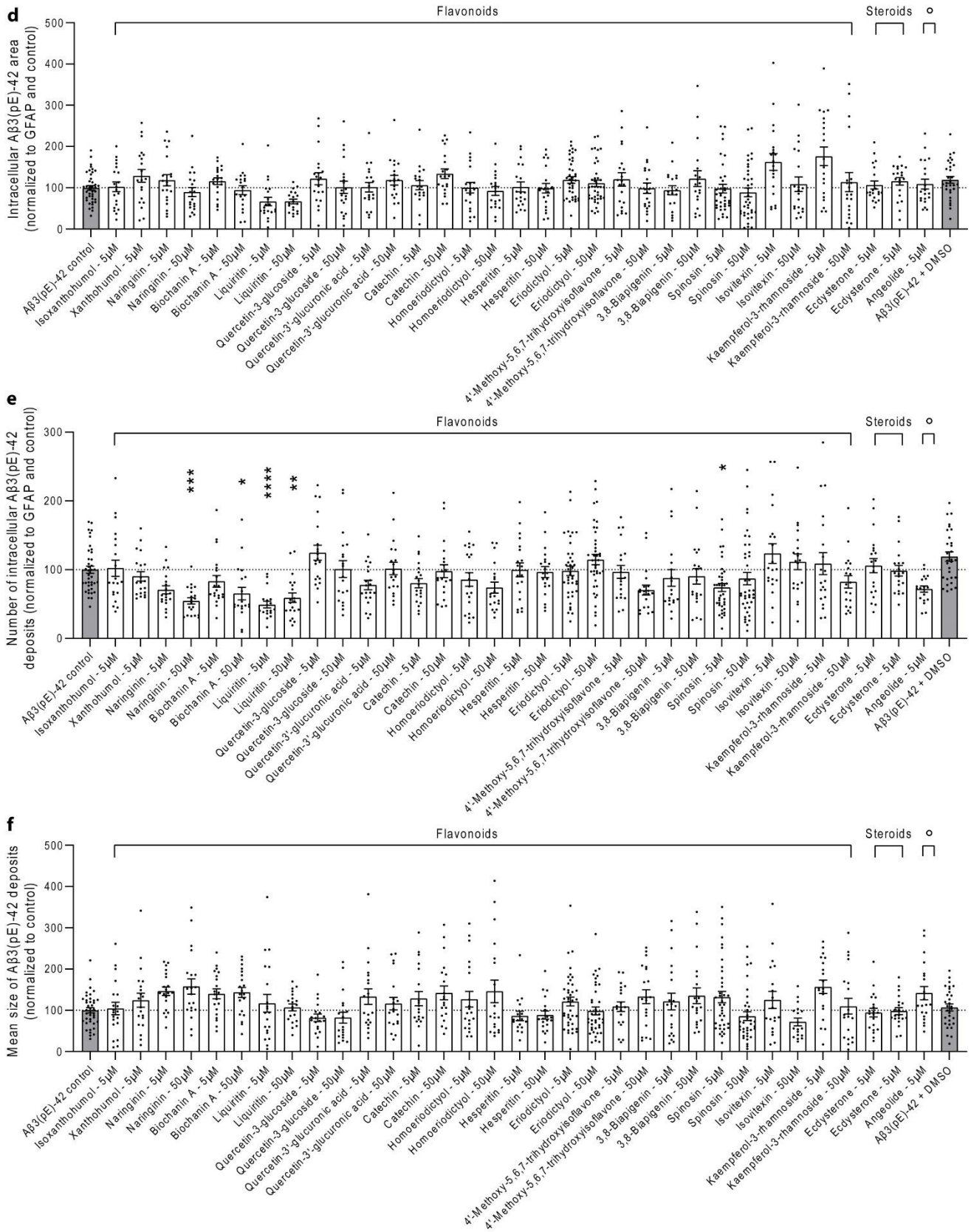
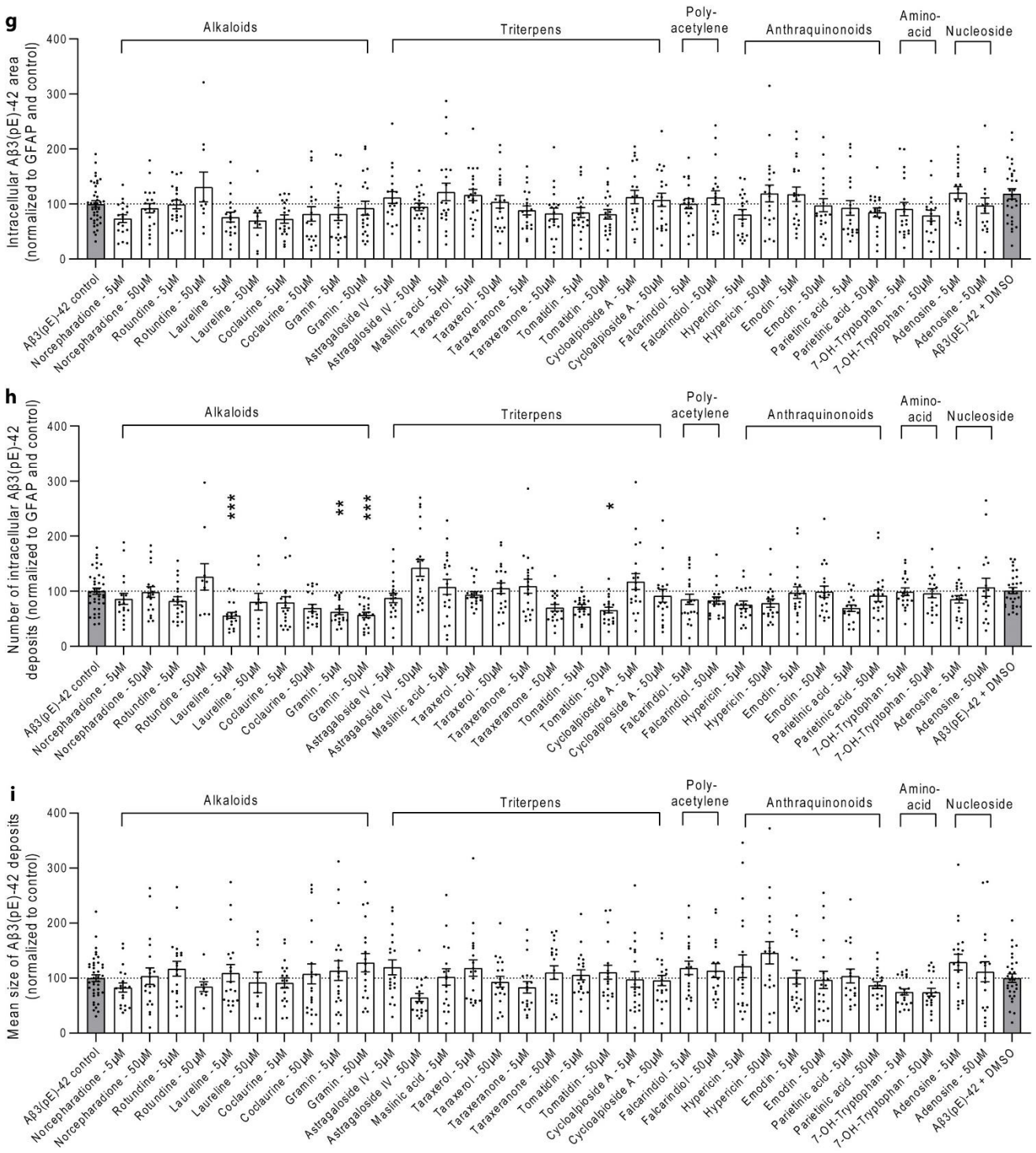
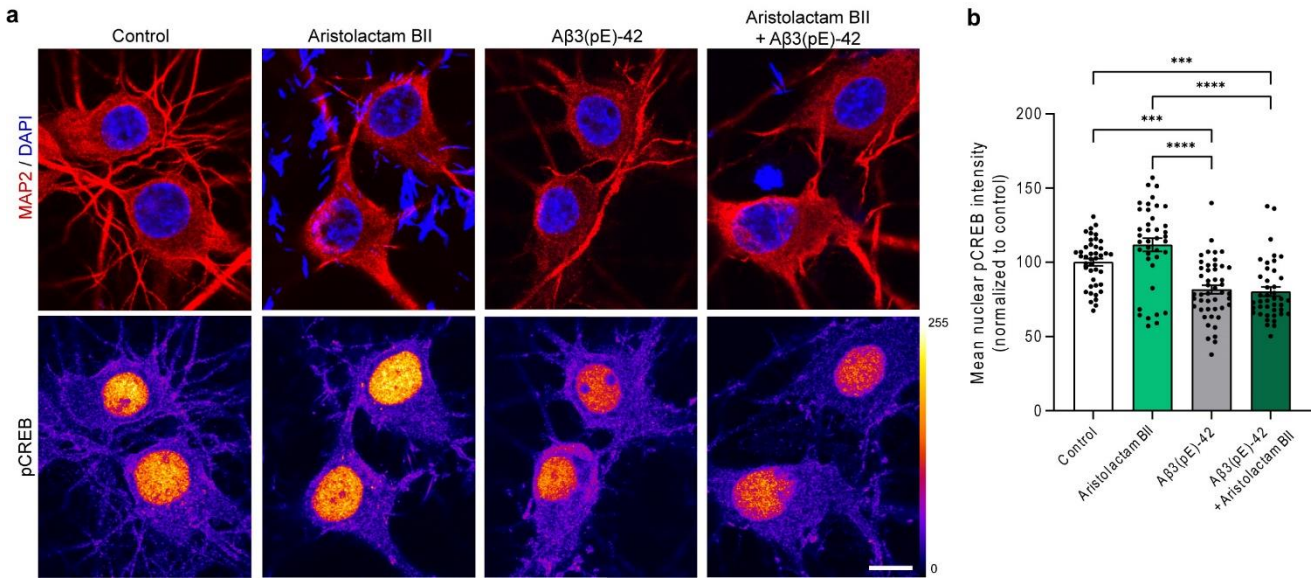
Chlorogenic acid - 50µM
Magnosalicine - 5µM
Magnosalicine - 50µM
Andamanicin - 5µM
Andamanicin - 50µM
Magnosalin - 5µM
Magnosalin - 50µM
Shikimic acid - 5µM
Shikimic acid - 50µM
Umbelliferone - 5µM
Umbelliferone - 50µM
Aβ3(pE)-42 + DMSO
c
Further phenolics
Lignans
Shikimic acid
Coumarin
Mean size of Aβ3(pE)-42 deposits (normalized to control)
400
300
200
100
0
Aβ3(pE)-42 control
Ferulic acid - 5µM
Ferulic acid - 50µM
Eicosyl ferulate - 5µM
Eicosyl ferulate - 50µM
Octyl ferulate - 5µM
Hexadecyl ferulate - 5µM
Hexadecyl ferulate - 50µM
Caffeic acid - 5µM
Caffeic acid - 50µM
Tyrosol derivative - 5µM
Tyrosol derivative - 50µM
trans-Asarone - 5µM
trans-Asarone - 50µM
Tenuifoliside A - 5µM
Tenuifoliside A - 50µM
Acteosid - 5µM
Acteosid - 50µM
Zingerone - 5µM
Zingerone - 50µM
p-Coumaric acid - 5µM
p-Coumaric acid - 50µM
Chlorogenic acid - 5µM
Chlorogenic acid - 50µM
Magnosalicine - 5µM
Magnosalicine - 50µM
Andamanicin - 5µM
Andamanicin - 50µM
Magnosalin - 5µM
Magnosalin - 50µM
Shikimic acid - 5µM
Shikimic acid - 50µM
Umbelliferone - 5µM
Umbelliferone 50µM
Aβ3(pE)-42 + DMSO


d
Intracellular Aβ3(pE)-42 area (normalized to GFAP and control)
Flavonoids
Steroids
e
Number of intracellular Aβ3(pE)-42 deposits (normalized to GFAP and control)
f
Mean size of Aβ3(pE)-42 deposits (normalized to control)
Aβ3(pE)-42 control
Isoxanthohumol - 5μM
Xanthohumol - 5μM
Naringinin - 5μM
Naringinin - 50μM
Biochanin A - 5μM
Biochanin A - 50μM
Liquiritin - 5μM
Liquiritin - 50μM
Quercetin-3-glucoside - 5μM
Quercetin-3-glucoside - 50μM
Quercetin-3'-glucuronic acid - 5μM
Quercetin-3'-glucuronic acid - 50μM
Catechin - 5μM
Catechin - 50μM
Homoeriodictyol - 5μM
Homoeriodictyol - 50μM
Hesperitin - 5μM
Hesperitin - 50μM
Eriodictyol - 5μM
Eriodictyol - 50μM
4'-Methoxy-5,6,7-trihydroxyisoflavone - 5μM
4'-Methoxy-5,6,7-trihydroxyisoflavone - 50μM
3,8-Biapigenin - 5μM
3,8-Biapigenin - 50μM
Spinosin - 5μM
Spinosin - 50μM
Isovitexin - 5μM
Isovitexin - 50μM
Kaempferol-3-rhamnoside - 5μM
Kaempferol-3-rhamnoside - 50μM
Ecdysterone - 5μM
Ecdysterone - 5μM
Angeolide - 5μM
Aβ3(pE)-42 + DMSO



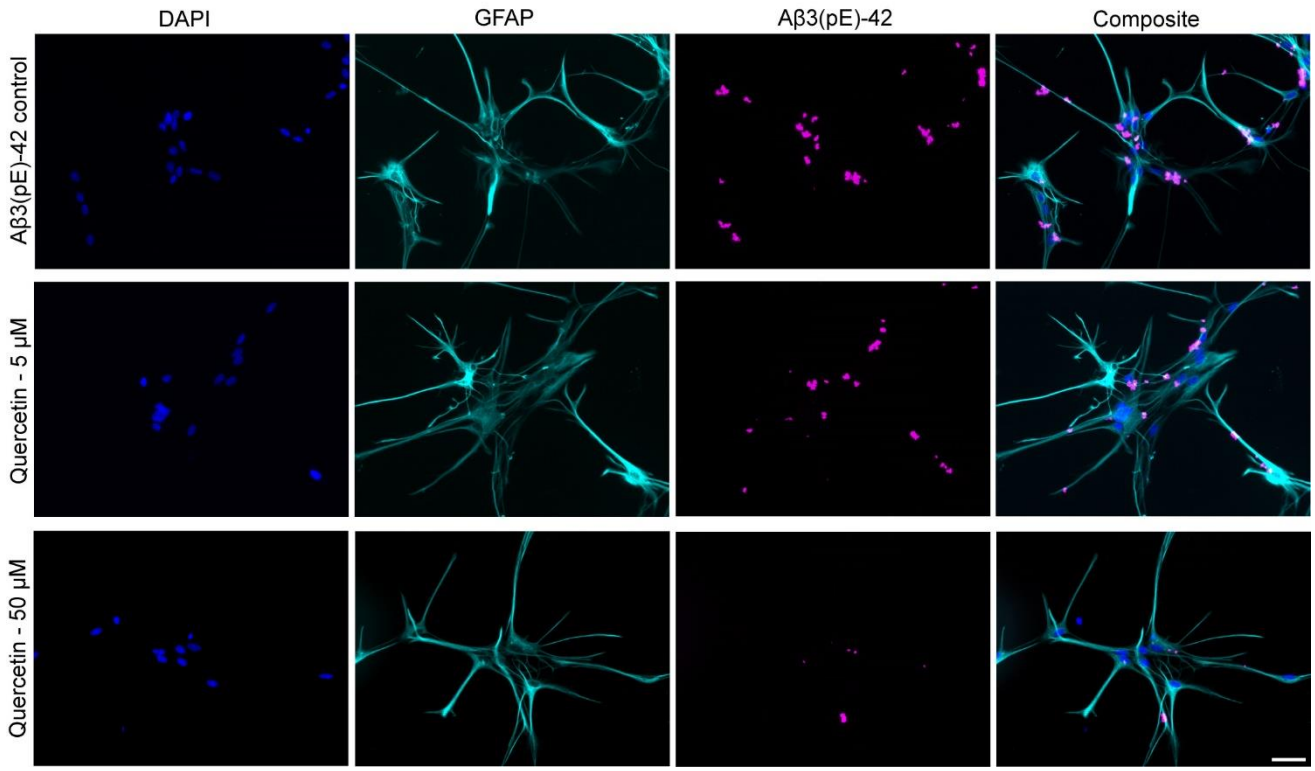
Supplementary Fig. 3 Plant substances that showed no major significant alterations regarding uptake of intracellular Aβ3(pE)-42 deposits in astrocyte cultures at the concentrations tested

(a-i) Control (incubation with Aβ3(pE)-42 only) and Aβ3(pE)-42 plus plant solvent DMSO are highlighted in grey. ° = Butylidenephthalide; Norcepharadione = Norcepharadione B; Taraxeranone = 14a-Taraxeran-3-one; 7-OH-Tryptophan = 7-Hydroxy-tryptophan; Biochanin A = 5,7-Dihydroxy -4'-methoxy-isoflavone; Ecdysterone = 20-Hydroxyecdysone; Isovitexin = Isovitexin-2"-O-b-D-glucopyranosid. *p<0.05, **p<0.01 by Kruskal-Wallis test followed by Dunn's multiple comparisons test. Data are presented as mean ± s.e.m.



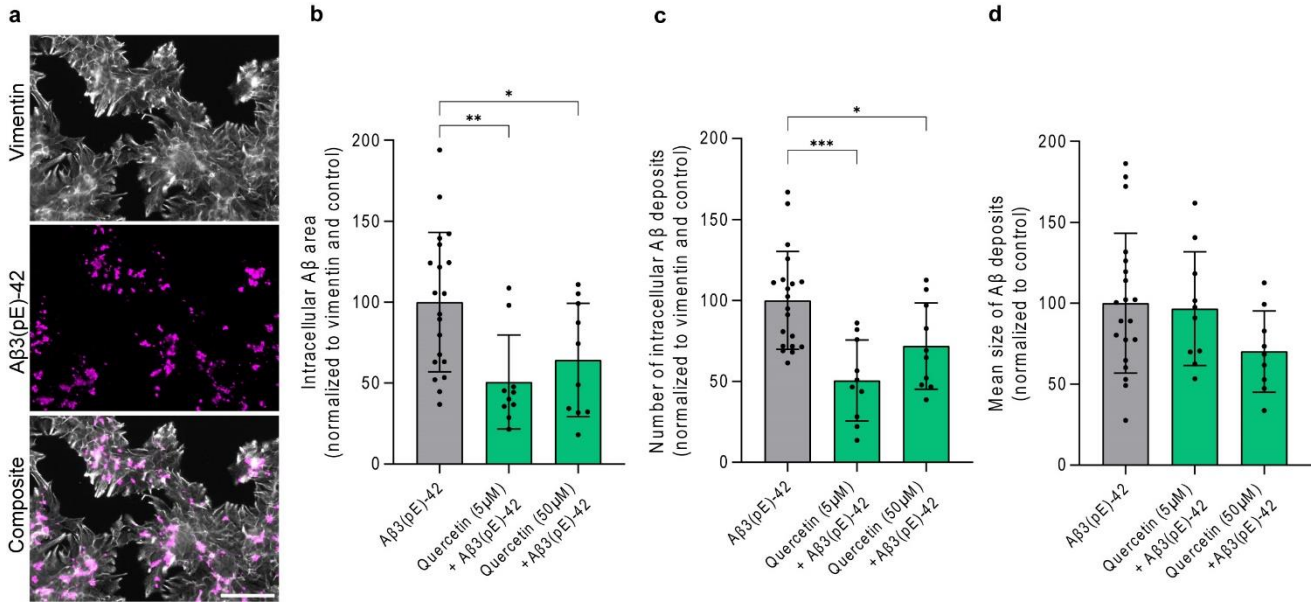
Supplementary Fig. 4 Aristolactam BII does not rescue Aβ3(pE)-42-induced CREB shutoff

(a) Representative confocal microscopy images of DIV18 neurons treated with 50 μM aristolactam BII, 500 nM Aβ3(pE)-42 oligomers or 50 μM aristolactam BII and 500 nM Aβ3(pE)-42 oligomers for 72h. Neurons were stained with MAP2 and pCREB antibodies and co-stained with DAPI. Scale bar is 10 μm. Lookup table indicates the pixel intensities from 0 to 255. **(b)** Mean pCREB fluorescence intensity within nuclear region (defined by DAPI) normalized to control. N= 39-47 nuclei from 2 independent cell cultures. ***p<0.001, ****p<0.0001 by Kruskal-Wallis followed by Dunn's multiple comparisons test. Data are presented as mean ± s.e.m.



Supplementary Fig. 5 Representative fluorescence microscopy images of primary astrocytes incubated with 500 nM Aβ3(pE)-42 only or 500 nM Aβ3(pE)-42 plus 5 µM or 50 µM quercetin for 16h.

Astrocytes were stained with GFAP and Aβ3(pE)-42 antibodies and co-stained with DAPI. Scale bar is 50 µm.



Supplementary Fig. 6 Quercetin reduces uptake and number of intracellular Aβ3(pE)-42 deposits in HEK-293T cell cultures

(a) Representative fluorescence microscopy images of HEK-293T cells treated for 10h with Aβ3(pE)-42 and stained with antibodies detecting Aβ3(pE)-42 and vimentin. Scale bar is 100 μm. **(b-d)** Treatment with both 5 μM and 50 μM quercetin reduces **(b)** area and **(c)** number but not **(d)** mean size of intracellular Aβ3(pE)-42 deposits. N = 10 FOVs. *p<0.05, **p<0.01, ***p<0.001 by one-way ANOVA followed by Dunnett's multiple comparison test. Data are presented as mean ± s.e.m.